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## Section B. Science

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# JOURNAL

OF THE

# MADRAS UNIVERSITY

## Section B. Science

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# THE DEVELOPMENT OF TEETH IN *SCOLIODON SORRAKOWAH* (CUV.)

BY

R. RAGHU PRASAD.

*(University Zoological Research Laboratory, Madras).*

## INTRODUCTION.

The teeth of all fishes and indeed of all higher vertebrates owe their origin to the modification of the dermal armour of the skin. In elasmobranchs during the inpushing of the outer skin in the formation of the stomodaeum the placoid elements are carried into the oral cavity. The identity in development and structure of placoid dermal elements with the teeth in elasmobranchs was probably first recognised by Williamson in 1849. Teeth are generally restricted to supporting bones or cartilages but unmodified placoid scales have been met with in elasmobranchs in the oral and pharyngeal cavities giving additional proof to the fact that the teeth in elasmobranchs are only modified placoid scales.

Many of the early biologists have shown considerable interest in the study of the structure and development of teeth in elasmobranchs and much work has already been done on this subject. Views differ widely on the mode of development of the parts of the teeth. Not only is there difference of opinion on their mode of development, but also there has been some confusion in terminology. The present work was done to study the mode of development of the different parts of the tooth in elasmobranchs in order to clear some of the doubtful points that at present exist. *Scoliodon sorrakowah* was selected as a type for the detailed study of the development of teeth, but other forms were also examined to compare the nature of the different parts and their mode of development. I take this opportunity to thank Dr. N. Kesava Panikkar, Director, University Zoological Research Laboratory for his kind help and suggestions.

## MATERIAL AND METHODS.

The material for the study included embryos of *S. sorrakowah* and also jaws of adults of *Hemigaleus balfouri, Rhynchobatus*

*djeddensis*, *S. sorrakowah*, *Carcharinus melanopterus*, *Astrape dipterygia*, *Narcine brunnea* and *Hypolophus sephen*.

The head region of the embryos of *S. sorrakowah* was fixed either in Sansom's fluid or Carnoy's. Sections of 7-10 microns thickness of the head and also of jaws of embryos were taken from material embedded in Paraffin. For the study of the teeth in other forms sections of jaws and also of individual tooth of adults were taken. Delafield's haematoxylin and Heidenhain's iron-haematoxylin were generally used for staining. For decalcification of the jaws of adults Nitric-acid-alcohol was employed.

## DEVELOPMENT OF TEETH.

The first step in development is that the ectoderm covering the jaws gets slightly invaginated just above the cartilage supporting the jaws. The ectoderm at this region thickens forming a solid lamina, the dental lamina, which dips down into the mesenchyme. This lamina is narrow at first but, as the embryo develops, the cells of the lamina proliferate and grow inwards.

The stage at which the dental lamina and tooth-germs appear varies from species to species. Thus Laaser (1903) observes that in *Spinax niger* and *Acanthias vulgaris* indication of the dental lamina is first seen in the lower jaw when the embryo is about 3·8 cm. long, though in the upper jaw it makes its appearance only a little later. In *Mustelus laevis* he notes that the dental lamina is better and earlier developed in the upper jaw than in the lower. Laaser (1903) quotes the observation of Treuenfels (1896) who remarks that in *Torpedo marmorata* dental lamina is seen in an embryo of 3 cm. and in slightly older embryos the formation of tooth-germs is seen.

The first sign of development of the tooth in *S. sorrakowah* is seen when the embryo is about 4 cm. long. Here also the first sign of development of dental lamina and tooth-germ is seen in the lower jaw as in *S. niger* and *A. vulgaris*. As the embryo grows, new tooth-germs are added on with the result that when the embryo is about 6 cm. there are about three rows of developing teeth in the lower jaw and in 11 cm. embryos there are five rows of teeth in the same jaw one of which is almost exposed. When the embryo is about to be liberated from the parent (13-14 cm. long) there are one or two rows of exposed teeth and another

four to five rows of reserve teeth in the lower jaw in various stages of development. The number of teeth formed at similar stages also varies according to species. A transverse section through the jaw of a young specimen gives the different stages in the development of teeth. In the present species the lower jaw carries a greater number of tooth-germs than the upper and in this respect also this form agrees with *A. vulgaris* and *S. niger* A fully developed elasmobranch tooth consists of two parts, a more or less transparent outer region, the 'enamel', and an inner layer, the dentine. Since in structure and development the 'enamel' in the teeth of elasmobranchs differs from the true enamel, I propose to use the term vitro-dentine as given by Owen (1849). Among the various types of dentine described (*Vide* page 9), Tomes (1898) remarks that in most elasmobranchs vaso-dentine is met with and in others osteo-dentine while in still others both these types are represented.

The youngest tooth-germ (Fig. 1) is a bluntly conical papilla consisting of ordinary mesenchyme cells covered over by an epithelium. This papilla is formed by the proliferation of the mesenchyme cells and as the papilla grows it takes along with it the epithelial layer which covers the papilla. The papilla is without specialised layers of cells at this stage and it resembles the corresponding stage in the development of the mammalian tooth. The epithelial cells at this stage are cubical and could hardly be differentiated from the other epithelial cells. In later stages they get separated from the surface of the mesenchyme cells leaving a space in between (Fig. 2), and are gradually transformed into a layer of columnar cells. At the same time the papilla gradually increases in size. These epiblastic columnar cells or ameloblasts are 12 μ long over the youngest papilla and have round deeply staining nuclei and feebly staining cytoplasm. These ameloblasts elongate (the largest being 22·8 μ) in later stages.

Simultaneous with these changes some modifications are noticed in the mesenchyme cells which lie beneath the ameloblasts. A layer of surface cells gradually become spindle shaped (Fig. 3) giving rise to long processes with a slight inclination outwards. These processes project into the space formed between the mesenchyme cells and the ameloblasts. The nuclei of these modified cells stain darkly and are almost round. The cytoplasm becomes slightly granular, gets more condensed and stains more

deeply. These cells are the 'osteoblasts' and they do not resemble the highly specialised, regularly arranged columnar odontoblasts of the mammalian tooth-germ. The layer thus formed by the prolongation of the tips of these modified mesenchyme cells is almost uniformly thick except at the base of the papilla where it begins to thin out. The layer thus formed by these prolongations of 'osteoblasts' is called the 'specialised layer' by Tomes (1898). I have retained the terms 'specialised layer' throughout this paper to mean that layer which is formed by the prolongations of the 'osteoblasts'. Figs. 4 and 5 show the specialised layer in the tooth-germ of *S. sorrakowah.* This specialised layer will be the site of formation of vitro-dentine. Thus by the formation of a layer of 'osteoblasts' the site of formation of vitrodentine is cut off from the rest of the pulp.

Where the specialised layer has reached its utmost development the ameloblasts have increased to their maximum size. The enamel organ remains as a continuous sheet, only specialised to a certain extent over each papilla. The epithelium which constitutes the enamel organ is continuous with the epithelium of the surface of the thecal fold. The large ameloblasts have their nuclei round and located in the middle of the cells. The ameloblasts in decalcified material show slight vacuolation. They have clear unbroken margins and their nuclei stain very lightly. These ameloblasts are also devoid of Tomes's processes which are invariably present along with the ameloblasts of mammals.

The range in the size of ameloblasts differs and, correspondingly, it has been noticed that the thickness of the vitro-dentine also differs. This is attributed to the shallowness of the thecal fold. Tomes (1898) says that wherever the thecal fold is shallow the ultimate thickness of the 'enamel' is less. Where there are well developed ameloblasts as in *S. sorrakowah, H. balfouri,* etc., there is a well developed layer of vitro-dentine but on the other hand in *N. brunnea* and *A. dipterygia* the ameloblasts are poorly developed and consequently the layer of vitro-dentine formed is also very thin.

The calcification of the papilla begins when it has been fully formed. It is supposed that the ameloblasts secrete the lime salts into the specialised layer, which is a matrix provided by the dentinal prolongations. The process of calcification is accompanied by changes in the ameloblasts. These tall ameloblasts suddenly

dwindle in size and the outlines of the individual cells are lost (Fig. 6). The perfect round nuclei of the ameloblasts become smaller and stain very poorly. The cytoplasm of these cells is stained somewhat darkly and they are no longer transparent. Thus it appears that these cells, when once they have performed their duty of providing the necessary lime salts, are no longer functional. Nunn (1882) does not believe that the enamel is formed as a result of the deposition of lime salts in the already existing dentinal matrix, but is of opinion that 'enamel', like dentine, owes its origin to the odontoblasts. Basing on my observations I am inclined to accept Tomes's (1898) view which I quote here. "It seems to be quite impossible to suppose that cells which undergo such enormous growth and other changes, can be without some important function, especially when it is found that these phenomena are constant in all the diverse genera which have been examined.

Yet it is equally certain that they do not fulfil quite the same part as the ameloblasts of Mammals, which, as is well established, furnish the whole thickness of enamel, both as respects its organic matrix and lime salts, which is accomplished after cutting off of this tissue from the dentine pulp by the formation of the skin of dentine, and they for a long time go on adding to the thickness of the enamel.

Such ameloblasts are invariably furnished with Tomes' processes, short in most Mammals and very long in Marsupials, but invariably present and essential to their function."

The specialised layer bears a direct proportion in its size to the thickness of the final vitro-dentine. Thus the vitro-dentine in elasmobranchs is partly mesodermal and partly ectodermal in origin and hence it is doubtful whether it merits the name enamel. The structure of the fully formed vitro-dentine is almost the same in all elasmobranchs except that in most rays it is a very thin layer. The penetration of vitro-dentine by the dentinal matrix is more or less common to all. Thus it is well marked in forms like *H. sephen, C. melanopterus* etc., and much reduced in others like *N. brunnea* and *A. dipterygia*. Forms like *S. sorrakowah* and *R. djeddensis* seem to bridge the two extremes. Towards the base of the tooth the layer of vitro-dentine becomes thinner and at the same time the number of dentinal prolongations is reduced and the layer of vitro-dentine presents a clearer appearance.

The vitro-dentine is hard and gives a brightly polished appearance to the surface of the tooth. The presence of lime salts can be demonstrated in a simple way. Sections of tooth are treated with dilute HCl (about 25%) for about 30 to 45 minutes. When these sections are examined it is noticed that the dentinal prolongations project freely and the lime salts which gave compactness to the layer, have been dissolved away. This could be well seen in those cases where there is a well developed layer of vitro-dentine with numerous dentinal prolongations traversing it. The vitro-dentine is supposed to contain Calcium fluoride combined with Calcium phosphate and carbonate to make it hard and resistant.

Beneath the specialised layer the modification of the pulp cells has already started. There is no regular arrangement of these dentine forming cells as in mammals. Along with the calcification of vitro-dentine, calcification of dentine also commences. Two theories have been advanced as to the mode of development of dentine. One is the conversion theory and the other the secretion theory, discussed in detail later on. The latter school of workers believes that the whole thickness of dentine is secreted by the odontoblasts whereas the former believes that the odontoblasts are themselves converted into the dentine. Of these two the conversion theory seems to have received a wider acceptance. Calcification begins and continues centrepetally. Tomes (1898) says "But the great and most striking peculiarity of these tooth germs lies in the fact that the first apparent calcification of true dentine, whether it be fine-tubed dentine as in *Carcharias*, or an osteodentine as in *Lamna*, takes place *not at the outside of the whole papilla, as invariably happens in Mammals, but at the inner side of the specialised layer*, thus cutting it off from the rest of the pulp".

The type of dentine met with in all the forms examined by me is osteo-dentine, because the dentine is not formed from the calcification of a "membrana eboris" or specialised layer of odontoblast cells, but by ossification of cells like osteoblasts. It may also be added that the dentinal tubes do not carry blood-vessels. It is an essential feature with vaso-dentine that the detinal tubes should carry blood-vessels. Thus the dentine is formed by the agglutination of the dentine forming cells, which are converted to form the whole thickness of dentine. These dentine forming cells are held together by an inter-cellular secretion which I believe is

probably secreted by the 'osteoblasts' themselves. The canal system is formed probably by the partial coalescence of the ossifying cells leaving interspaces between them. The size and number of canals traversing the dentine vary from species to species. Thus in *S. sorrakowah* they are few whereas in *C. melanopterus* these are numerous.

Usually the pulp cavity remains undivided but in *H. sephen* the pulp cavity is divided into many chambers. This is effected by the growth of dentine into the pulp cavity, the growth being not regular but interrupted.

## DISCUSSION.

The teeth of elasmobranchs consist of two parts viz., the outer transparent layer known usually by two different terms, enamel and vitro-dentine, and the inner dentine. The question whether the elasmobranch teeth have enamel at all has been answered variously by different authors. M'Coy (1848) recognised the fact that enamel of fishes differs in development from that of mammals and first applied the term 'ganoine' for the layer of false enamel on the teeth of certain fossil sharks. Owen (1849), however, pointed out that he had already used the term vitro-dentine. Williamson (1849) independently used the term, 'ganoine' for the enamel-like substance in the scales of *Lepidosteus*. Röse (1897) considers the inner portion of the 'enamel', where the dentinal prolongations are numerous, as ordinary dentine and its outer portion as vitro-dentine where the layer presents a clearer appearance because the dentinal prolongations do not extend up to this region. Jentsch (1897), as quoted by Laaser (1903), calls this particular layer vitro-dentine. Tomes (1898) retains the term enamel and suggests that just as the entire teeth of Selachians present the problem of tooth formation reduced to its simplest aspect, so this layer appears to be the first introduction of enamel as a separate tissue, and therefore, to avoid multiplication of terms it may be appropriately called enamel. He divides enamel into the following types :—

1. Enamel not wholly epiblastic in origin. The stroma which is the seat of enamel calcification is furnished by the transformation of the exterior of the mesoblastic dentine papillae, the ameloblasts apparently secreting the lime salts required for its hardening. This type is found in elasmobranchs.

2. Enamels wholly epiblastic in origin:—

(a) In which the ameloblasts undergo a prior transformation into a stroma of the dimensions of the furnished product and themselves disappear. This type is met with in Gadidae—in *Sargus* and *Labrus* and probably other fishes.

(b & c) Also epiblastic in origin but in which the ameloblasts persist. This type is confined to the mammals.

Ritter (1900), as quoted by Laaser (1903), calls the outermost layer on the teeth of *Trygon* and *Acanthias* by the term enamel. Laaser in the same paper says that Hertwig also maintained this layer as enamel. Personally he is inclined to call this vitrodentine. Jacobshagen (1923) seems to retain the term enamel.

Many of the discussions as to the nature of the 'enamel' and dentine in elasmobranchs have relation to placoid scales rather than to teeth. A detailed summary of the various views on the nature and formation of 'enamel' has been given by Tomes (1898) and these details are therefore not repeated. From my study, I find that the so-called enamel is formed as a joint product of two different tissues viz., the outer mesoblastic cells of the dentine papilla and the epiblastic ameloblasts. The matrix of the ultimate 'enamel' in its full thickness is laid down by the prolongations of the dentine forming cells and into this matrix the highly modified ameloblasts secrete the mineral substance. Thus in development and structure the 'enamel' of the elasmobranchs differs from the true enamel. Hence to avoid confusing this layer with true enamel, I feel that the term vitro-dentine, suggested by Owen, may be retained and this term is particularly suitable since the entire 'enamel' in elasmobranchs is unquestionably formed in the matrix provided by the prolongations of the 'osteoblasts', while the ameloblasts play only a minor role i.e., secretion of mineral substance.

The dentine was accurately described, as early as 1836, by Retzius. The various types of dentine were named by Owen (1840-'45), in his treatise on 'Odontography'. Later on Tomes (1878) defined more precisely the different types and the forms now generally recognised are:—

(1) *Hard unvascular dentine.*

(2) *Vaso-dentine,* which is developed from odontoblasts after the manner of dentine, but contains an anastomosing net-work of canals modelled around and containing capillaries.

(3) *Plici-dentine,* developed from odontoblasts, but from a complicated pulp so that it is more or less divided up into distinct systems of dentinal tubes.

(4) *Osteo-dentine,* developed from osteoblasts, like bone, and quite unlike dentine but permeated by a system of large canals, which do not contain or have any special relation to blood-vessels.

Röse (1897) uses the term 'trabicular dentine' for vaso-dentine and Burckhardt (1906) uses the term 'trabiculin' for the same layer.

Tomes (1878) says: "Thus all of the Rays examined have dentine of fine-tubed variety, as have also the transitional *Rhina squatina, Carcharias, Scymnus, Galeus, Acanthias* and *Scyllium*".

*Lamnidae,* on the other hand, have all dentine of osteodentine type." He also remarks that it is likely that the upstanding part of the tooth is composed of purely fine-tubed dentine and the base osteodentine. As far as I could see in all the specimens studied by me it is osteodentine.

Two main views have been held on the formation of bone. According to one view the mass of individual osteoblasts is consolidated by deposit of lime salts and the laminae of the bone are made up of these layers of consolidated cells. The bone corpuscles are the osteoblasts, which have become included in the calcified matrix, but have themselves remained uncalcified. According to the other view osteoblasts secrete a substance which calcifies, and this secretion being not uninterrupted, but intermittent, the laminae of the bone are produced. These two views, with some modifications have also been held as to the formation of dentine. Details of these views have been given by Mummery (1893).

Based on my observations I am led to conclude that the dentine in elasmobranchs is formed not only by the conversion of the specialised mesoblastic cells but that these mesoblastic cells while getting specialised secrete the necessary lime salts which help in consolidating these modified cells and giving them rigidity. So

both conversion as well as secretion take place in the formation of dentine.

## SUMMARY.

1. The development of teeth in *S. sorrakowah* is described.

2. The term vitro-dentine is suggested in place of enamel because the outer layer (vitro-dentine) is a joint product of mesoblastic and epiblastic cells. It is certainly not dentine but while its organic matrix is beyond question furnished by the modified mesoblastic cells of the dentine papilla, the ameloblasts are responsible for the organic salts which give the layer its hardness.

3. Mode of development of vitro-dentine is the same in all forms examined though in thickness and structure the vitro-dentine varies from species to species. In structure it ranges from the narrow layer in *N. brunnea* and *A. dipterygia* with only a few dentinal prolongations traversing it to the thick layer in *C. melanopterus* and *H. sephen* with numerous dentinal prolongations.

4. Dentine, in all forms examined, is of the osteo-dentine type. It is formed both by conversion as well as by secretion. While the mesoblastic cells get converted into 'osteoblasts' they secrete lime salts which agglutinate them and give compactness. Hence either conversion or secretion theory alone cannot be maintained for explaining the formation of dentine.

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N.B:—References marked with an asterisk have not been referred to in the original. Complete list of earlier references may be found in Laaser (1903) and Burckhardt (1906).


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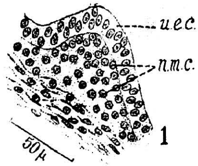
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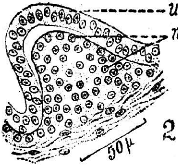
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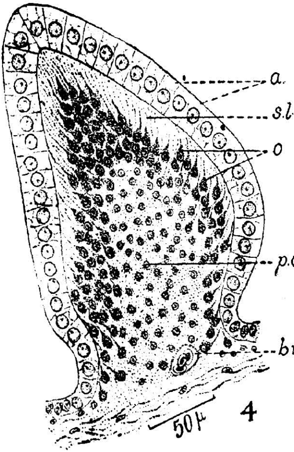
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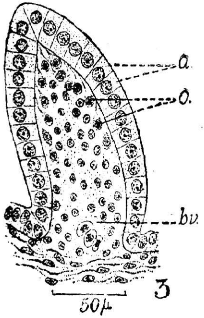
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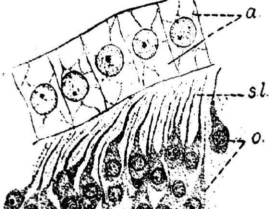
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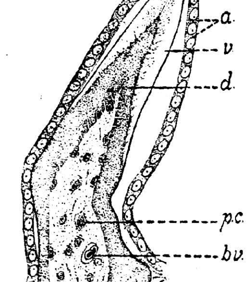
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p.c.
b.v.
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## EXPLANATION OF FIGURES.

Fig. 1. A blunt conical tooth-germ where no specialisation of layers has taken place.

Fig. 2. A slightly advanced papilla. Note the space formed between the ectodermal cells and mesenchyme cells.

Fig. 3. A stage showing the onset of specialisation. Ectodermal cells are modified into ameloblasts and a few of the mesenchyme cells at the tip of the papilla show modification into the 'osteoblasts'.

Fig. 4. A fully developed papilla with the different regions well formed. Note the fully developed ameloblasts, numerous 'osteoblasts', and the well developed specialised layer.

Fig. 5. The specialised layer is shown enlarged.

Fig. 6. Section of a tooth showing dentine and vitro-dentine. Notice the highly reduced ameloblasts.

## KEY TO LETTERING.

| | |
|---|---|
| a. | ameloblasts. |
| bv. | blood-vessel. |
| d. | dentine (osteo-dentine type). |
| n.m.c. | nuclei of the mesenchyme cells. |
| o. | 'osteoblasts'. |
| p.c. | pulp cell. |
| s.l. | specialised layer. |
| u.e.c. | unmodified ectodermal cells. |
| v. | vitro-dentine. |

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# SEASONAL VARIATION IN INFANT MORTALITY

By

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A scientific study of Infant Mortality is of immense interest especially in a country like India with its high death-rate. The registration of births and deaths, imperfect as it is, prevails only in urban areas and as such a critical study of seasonal variation can be conducted only on a regional basis. In this paper a study of infant mortality in the City of Madras is made for a period of 15 years (1926-1940) with a special stress on the extent to which the infant deaths respond to local climatic changes.

Infant deaths below one year are taken from the weekly reports published by the Corporation of Madras as the primary data for the study. The total number of live births are also noted for compiling the mortality rate. At the outset it may be pointed that there are possibilities of errors and omissions in the records published. The recorded figures may fall short of the actuals and the age of the child specified may not be accurate. Further, some deaths occuring in hospitals may not relate to ordinary residents of Madras. But as the study extends over a period of 15 years, the trend and the seasonal variation noted can be taken to be fairly accurate.

In this study the year is divided into 13 months of equal duration of four weeks each.[1] By adopting this procedure a day in the year has to be left out. But this has the advantage of having equal duration for all months, and further as no accurate figures are available for calendar months, this method is adopted.[2]

1. 'Months' in this study correspond to the calender months of the civil year in the following manner:—

| | | | |
|---|---|---|---|
| 1. | From 1st Jan. to 27th Jan. | 8. | From 16th July to 12th Aug. |
| 2. | ,, 28th Jan. to 25th Feb. | 9. | ,, 13th Aug. to 9th Sep. |
| 3. | ,, 26th Feb. to 25th Mar. | 10. | ,, 10th Sep. to 7th Oct. |
| 4. | ,, 26th Mar. to 22nd Apr. | 11. | ,, 8th Oct. to 4th Nov. |
| 5. | ,, 23rd Apr. to 20th May. | 12. | ,, 5th Nov. to 2nd Dec. |
| 6. | ,, 21st May to 17th June. | 13. | ,, 3rd Dec. to 30th Dec. |
| 7. | ,, 18th June to 15th July. | | |

2. The same procedure has been adopted in "The influence of seasons on human production"—N. T. Mathew. Sankkya. Vol. V. Pp. 261-268.

TABLE I

*Monthly figures of Infant Mortality Rate in the City of Madras*

| Year. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | Average. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1926 | 321·89 | 295·36 | 276·50 | 252·32 | 255·86 | 326·61 | 242·30 | 334·27 | 294·77 | 285·93 | 224·62 | 261·17 | 259·40 | 279·31 |
| 1927 | 215·70 | 182·27 | 183·84 | 213·09 | 242·86 | 208·39 | 202·12 | 352·01 | 209·43 | 185·41 | 221·45 | 300·83 | 328·51 | 234·30 |
| 1928 | 354·63 | 265·02 | 220·21 | 234·42 | 280·86 | 274·31 | 221·81 | 344·25 | 365·56 | 297·26 | 306·00 | 299·99 | 264·47 | 286·83 |
| 1929 | 274·79 | 260·71 | 251·70 | 305·19 | 242·69 | 214·54 | 233·68 | 249·45 | 225·80 | 242·13 | 235·93 | 331·10 | 313·08 | 260·06 |
| 1930 | 260·70 | 226·28 | 224·25 | 220·71 | 221·21 | 249·06 | 216·66 | 223·75 | 196·41 | 212·61 | 218·68 | 346·25 | 332·07 | 242·20 |
| 1931 | 329·96 | 273·40 | 233·21 | 195·99 | 235·19 | 287·20 | 241·15 | 216·34 | 284·31 | 198·97 | 194·50 | 226·76 | 273·89 | 245·45 |
| 1932 | 242·94 | 193·79 | 189·16 | 191·94 | 208·63 | 247·11 | 199·82 | 208·17 | 227·64 | 267·51 | 295·79 | 293·47 | 277·71 | 234·13 |
| 1933 | 286·20 | 269·28 | 257·39 | 277·51 | 256·48 | 226·76 | 216·70 | 255·57 | 228·13 | 240·94 | 273·40 | 341·06 | 321·86 | 265·48 |
| 1934 | 278·59 | 253·64 | 240·24 | 206·52 | 223·15 | 253·18 | 205·59 | 263·81 | 201·90 | 228·69 | 194·51 | 225·00 | 212·99 | 229·83 |
| 1935 | 231·34 | 186·59 | 171·82 | 178·15 | 194·19 | 241·90 | 204·75 | 257·52 | 232·61 | 220·79 | 295·09 | 275·67 | 207·28 | 222·90 |
| 1936 | 221·31 | 188·98 | 165·19 | 196·62 | 191·23 | 202·45 | 206·94 | 244·65 | 225·35 | 210·98 | 206·49 | 233·43 | 289·54 | 214·09 |
| 1937 | 268·73 | 190·04 | 179·15 | 182·05 | 196·74 | 224·78 | 182·92 | 218·92 | 230·22 | 206·36 | 224·36 | 267·89 | 291·75 | 220·30 |
| 1938 | 292·87 | 245·95 | 213·27 | 177·23 | 219·97 | 176·81 | 243·85 | 248·04 | 193·57 | 230·44 | 253·49 | 221·65 | 198·60 | 224·29 |
| 1939 | 192·81 | 227·08 | 200·24 | 199·83 | 234·51 | 238·23 | 184·55 | 254·74 | 219·65 | 198·59 | 317·08 | 329·88 | 265·89 | 235·62 |
| 1940 | 225·14 | 177·43 | 156·53 | 182·95 | 197·54 | 222·38 | 184·53 | 191·62 | 204·24 | 189·26 | 178·22 | 218·43 | 292·95 | 201·63 |
| Average. | 266·51 | 229·05 | 210·85 | 214·30 | 226·74 | 239·58 | 212·49 | 257·54 | 235·97 | 227·72 | 242·64 | 278·17 | 275·33 | 239·76 |

'Infant mortality is usually computed by relating the number of deaths under one year to the number of births of the same calendar year'.[3] This will be a correct estimate only if all the infant deaths during the year are from the births in that year. But in fact a certain portion of the deaths belongs to infants born in the preceding year. So a correction for this has to be made to get at the accurate infant mortality rate. But as no proper data is available, and in view of the fact that most of the infant deaths occur only in the first few months of the child's birth, crude mortality rate is utilised for this study.[4]

The infant mortality rate for any month is taken as the number of infant deaths per thousand of live births in that year, assuming the same death-rate to continue throughout the year. Table I gives the monthly rates for the 15 years. The last row and the last column indicate respectively the mean monthly and yearly mortality rates.

The average mortality rate for the period under consideration is 239·76 which is very high constituting as it is a quarter of the total live births. The yearly averages in the last column indicate a decreasing tendency. A second degree polynomial is fitted[5] to these averages to show the trend. The parabolic trend together with the yearly averages is indicated in Graph I.

$y = 239{\cdot}761 - 3{\cdot}0761\ \xi_1 + 0{\cdot}0625\ \xi_2$ where $\xi_2 = (\xi_1^2 - 4)$ and $\xi_1 = (x - 1933)$, x any year and y the corresponding mortality rate.

Although year to year averages exhibit an appreciable degree of variation. the general trend is almost linear and indicates a rapid fall in infant mortality. The usual method of analysis of variance is adopted to separate the variation due to years and months. The significance of the variance is tested by using the ratio of mean square variation to mean square interaction.[6]

3. See p. 27—R. R. Kuczynski 'Population Movements' (1938).

4. "Since actually mortality is much higher at the beginning than at the end of the first year of age the majority of infants deceased in a calender year have been born in this calender year, and a small minority in the proceeding one, the ratio as a rule being 7: 3". ("The Measurement of Population Growth. Methods and Results", R. R. Kuczynski—p. 169). But this ratio will be still high for Indian data and as no recognised ratio is available for Indian conditons crude mortality rate can be taken.

5. R. A. Fisher, 'Statistical Methods for Research Workers'—p. 148.

6. C. W. Sredecor—'Calculation and interpretation of analysis of variance and covariance' (1935).

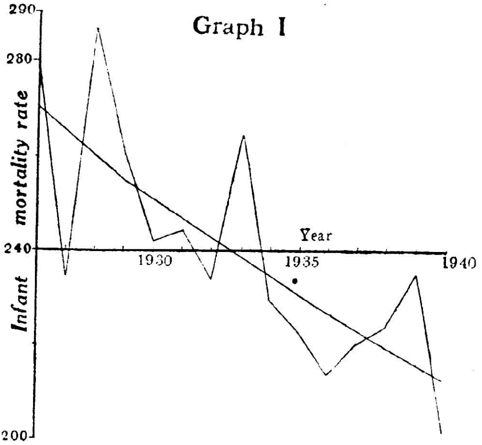



TABLE II

| Source. | D. F. | Sum of squares. | Mean square. | Ratio 'F'. | 5% | 1% |
|---|---|---|---|---|---|---|
| Between means of years. | 14 | 105,143·77 | 7,510·27 | 10·152 | 1·781 | 2·233 |
| Between means of months. | 12 | 97,759·42 | 8,146·62 | 11·013 | 1·813 | 2·300 |
| Interaction. | 168 | 124,277·96 | 739·75 | | | |
| Total. | 194 | 327,181·15 | 1,686·50 | | | |

The high values abtained compared with the 1% level indicates the existence of significant variation in years and also in months. The mean square variation for months is even higher than the mean square variation for years which indicates the presence of prominent seasonal factor in infant deaths.

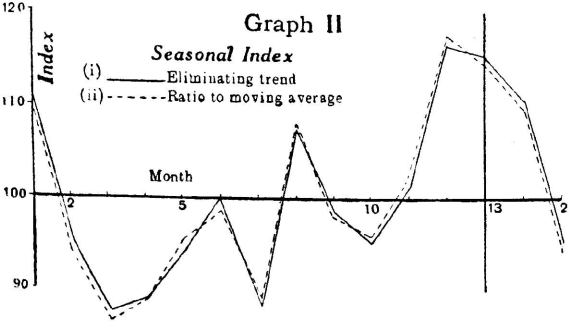
The fluctuations in any time series can be taken to consist of secular trend, seasonal, cyclic and residual variations. The last two can be eliminated by taking the average figures for a fairly long period. The monthly averages given in the last row of table I can be taken to be free of these two types of fluctuations. So the seasonal indices can be formed by eliminating the secular trend from these averages.

3

Secular trend can be eliminated with the help of the polynomial fitted to the yearly averages. As the linear trend is prominent, necessary corrections are made in the monthly averages to adjust to the straight line trend.[7] The corresponding monthly averages adjusted to trend are shown in column (3) of table III. Column (4) gives the seasonal indices making the total 1300 for all the months and they are indicated in Graph II.

TABLE III

| Month | Monthly average | Adjusted for trend | Adjusted for total | Ratio to moving average | Corrected seasonal Index |
|---|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 | 6 |
| 1 | 266·51 | 266·510 | 110·501 | 110·126 | 109·630 |
| 2 | 229·05 | 229·287 | 95·068 | 94·460 | 94·034 |
| 3 | 210·85 | 211·324 | 87·620 | 87·042 | 86·650 |
| 4 | 214·30 | 215·011 | 89·149 | 89·428 | 89·025 |
| 5 | 226·74 | 227·688 | 94·405 | 95·745 | 95·313 |
| 6 | 239·58 | 240·765 | 99·827 | 99·442 | 98·994 |
| 7 | 212·49 | 213·912 | 88·693 | 89·692 | 89·288 |
| 8 | 257·54 | 259·199 | 107·470 | 108·551 | 108·062 |
| 9 | 235·97 | 237·866 | 98·625 | 98·756 | 98·311 |
| 10 | 227·72 | 229·853 | 95·302 | 96·411 | 95·976 |
| 11 | 242·64 | 245·010 | 101·587 | 103·170 | 102·705 |
| 12 | 278·17 | 280·777 | 116·417 | 118·208 | 117·675 |
| 13 | 275·33 | 278·174 | 115·337 | 114·856 | 114·338 |




7. The trend factor to be eliminated is—3·0761/13=0·237 per month. This amount should be added to the second month; twice this to the third month and so on to get the corrected monthly averages.

Seasonal indices may also be obtained by forming the ratio to moving averages. 'For stable seasonals the logarithmic moving average is the most appropriate method'.[8] This method consists in finding out the arithmetic mean of the deviations for a number of years from the logarithmic moving average. Here linearity is assumed only for the logarithms and the seasonals are taken not to change during the period chosen for study. This method is applied to the infant mortality rates to form the seasonal indices. The algebraic proof for working out the 13 months moving averages is given elsewhere.[9] Column (5) of table III gives the seasonals formed by this method and column (6) the indices after a slight adjustment for the total. The indices in column (6) are also shown in graph II.

Both the methods indicate the same tendencies in seasonal variation except for the slight differences in intensity. The graph indicates three troughs in 3rd, 7th and 10th months and three peaks in the 6th, 8th and 12th months. Taking the calendar months the minimum points are in March, in the later half of June to middle of July, and in September. The maximum points are in the later part of May and the early half of June, in later half of July extending to August, and in November. The peaks noted in the 6th and 8th months are not prominent. So, in general, infant mortality can be taken to be below normal from 2nd to 11th months (beginning of February to October) and above the normal level from 11th to 2nd month (from October to the end of January).

This prominent seasonal variation in infant mortality can be explained by the local climatic changes of the City of Madras. The North East monsoon is responsible for rainfall during the months of October and November though stray showers are common even in September and December. Then sets in winter which lasts till the end of January. The damp climate supported by conditions that lead to the spread of diseases may be taken to aid infant mortality which reaches the maximum in the early part of December. Later the slow change in the weather seems to aid the fall in mortality till the end of March. Increasing tendency is again visible till the middle of June, though the variation is below the

8. 'The seasonal factor in Indian Trade, Industry and Finance',—Sinha-Sankhya Vol. VI. Part (1) page 53 (1942).

9. 'Mortality in the city of Madras. A study of Seasonal Variation 1926-40'—Journal of the Madras University (1944) Pp. 207-213.

normal level. This may be due to the extreme hot weather, which the infants usually cannot bear. Then there is a fall in the first half of July followed by a steep rise in the next month. This can be attributed to the few showers that are not uncommon in July. After the middle of August there is a steady fall till October, aided by the moderate and rather dry climate. So, in general, the seasonal variation appears to be in tune with the local climatic changes.

In this brief survey a steady decreasing tendency is noticed in infant mortality in the City of Madras, though yearly variation is prominent. The seasonal variation is well marked, with the minimum in the early part of April and the maximum in the month of December. This variation can be explained by local climatic changes.

# NITROGEN METABOLISM AND HUMAN NUTRITION

BY

MANAYATH DAMODARAN.

(*Biochemical Laboratory, University of Madras*).

## Part III

## THE PROTEIN REQUIREMENTS OF FARM ANIMALS

*In order to determine the actual net nutrients required to produce a given animal product, the composition of the product should be known, as well as the composition of the available nutrients in food which is to be fed for its production so that the nutrients in the ration might be provided in the proportions needed by the animal. Before a builder builds on a contract he determines the quantity needed of each of the materials that are to appear in the structure. Without such specifications he would not know how much of each of the different materials would have to be provided.*

HAECKER.

The farm animal needs to be supplied in its food not only the materials required by it for the maintenance of normal health but also those required for the manufacture of those products which the human being expects to derive from it—meat or milk, hide or wool. For this reason the assessment of the protein requirements of farm stock is beset with even greater difficulties than in the case of human beings. The uncertainty attached to the determination of biological values is greatly increased by the necessity for taking into account the additional requirements for meat or milk production as also by the large variety of feeding stuffs.

The data available have been obtained from three types of experiments: (i) From nitrogen balance studies using the formula of Thomas or one of its modifications, (ii) from the energy metabolism, on the assumption that endogenous nitrogen metabolism bears a constant relation to basal metabolism, (iii) from long period feed trials. These different methods have not only yielded widely different results but the same method in the hands of different workers has not given concordant results.

***Protein Requirements for Maintenance.*** The difficulties in the determination of biological value of proteins by the formula of Thomas have already been mentioned. With dairy animals these are greatly increased not only because of the impossibility of separating utilization for maintenance from utilization for production but also because the minimum endogenous catabolism, i.e., the balance on a nitrogen-free diet, cannot be determined with any certainty as it is impossible to make animals eat sufficient quantities of nitrogen-free food to satisfy their energy requirements for any length of time. Mitchell who carried out extensive investigations on the biological value of proteins in farm feeds for "maintenance" came to the conclusion that all American feeds have a biological value of about 50%. On the assumption that the protein requirement for maintenance should just suffice to balance the endogenous catabolism, which according to his estimations amounts to 0·03 g. N per Kg. body weight, Mitchell concludes that an intake of digestible protein corresponding to 0·06 g. N per Kg. is a suitable maintenance ration. This works out to 0·4 lb. for an animal of 1000 lb. body weight and is considerably below the standards generally used in practical animal husbandry.

That a constant relationship exists between basal metabolism and endogenous nitrogen metabolism has been suggested by several authors. Brody and his associates who investigated the energy metabolism of animals varying in size from mice to elephants came to the conclusion that for all the species studied the basal metabolism Q in calories is related to the body weight M in kilograms according to the following equation:

$$Q=70 \cdot 5 \times M^{0 \cdot 734}$$

Brody further showed that the endogenous urinary nitrogen also tends to vary with the same power of body weight as the basal metabolism. According to Smuts 2 mg. of nitrogen or 12·5 mg. of protein is to be considered equivalent to 1 calorie of basal heat so that protein requirement can be calculated from body weight from the equation:

$$P=0 \cdot 88\ M^{0 \cdot 734}$$

where P is the day's protein requirement in grams and M the body weight in kilograms. Assuming with Mitchell a biological value of 50% for feed protein the above formula works out to 0·35 lb. of digestible protein as the requirement for a 1000 lb.

animal. Brodie himself considers that both energy and protein requirements are twice those indicated by the basal metabolism and recommends 0·70 lb. per kilogram. The only justification for thus doubling the value appears to be that the figure so obtained is in closer agreement with the requirements indicated by feed trials.

*Protein Requirements for Milk Production.* The nitrogen balance method has been used by several authors, notably Mitchell, Maynard and Hart to assay the biological value of proteins for growth and milk production, the percentage of the absorbed nitrogen which was retained by the animal being taken as a measure of its efficiency for growth and the percentage which was secreted in the milk as an index of its value for milk production. Their painstaking experiments have not yielded much quantitative data of general applicability, but have enabled comparisons to be made of the relative efficiencies of proteins from different sources fed under strictly defined conditions. The results obtained by Morris and Wright on the biological values for milk production of various feeds used as supplements to a basal ration of oats, beet pulp and straw are given below:

TABLE I.

*Biological Value of Feeds for Milk Production*

| Feed. | Biological Value. |
|---|---|
| Fresh and dried spring grass, Grass silage (made from summer grass), Low-temperature dried-blood meal. .. | 75 to 80 per cent |
| Fresh and dried autumn grass, Bean and pea meals. .. | 60 to 65 per cent |
| High-temperature dried-blood meal, Meat meal. .. | 55 to 60 per cent |
| Decorticated earth-nut cake, Decorticated earth-nut cake plus flaked maize. .. | 50 to 55 per cent |
| Linseed cake, Linseed-oil meal. .. | 45 to 50 per cent |

The net result of the large amount of work done on these lines is best summed up in the words of Maynard: "These various studies of protein quality which have been cited serve to emphasize the uncertainties and difficulties of the measure used rather than providing much information which bears on the nutritive require-

ments in lactation or which can serve as a guide for the selection of efficient combinations in practice".

*Amino-acid Requirements.* For further progress in this field it is necessary to gain universally applicable fundamental knowledge regarding amino-acid requirements which would enable the efficacy of feeds to be predicted from chemical analysis.

Rose's experiments on the amino-acids essential for growth have already been mentioned in connection with human nutrition. It remains to consider the rather meagre knowledge available at present in relation to other functions.

Investigations carried out by Wright and collaborators on the nutritional efficiency of feeds in relation to their amnio-acid composition make it probable that in many common feeds lysine is the limiting factor in milk production.

TABLE II.

*Lysine Nitrogen Content of Milk and Feeds*

| | | | |
|---|---|---|---|
| Skim milk | 8·27% | Cottonseed meal | 4·21% |
| Blood meal | 10·38 | Linseed meal | 3·51 |
| Meat meal | 8·14 | Oats | 2·91 |
| Beans | 7·44 | Red clover hay | 2·62 |
| Peas | 7·04 | Wheat | 2·47 |
| Soyabeans | 6·18 | Corn | 2·17 |
| Peanut cake | 4·46 | Barley | 2·19 |
| Alfalfa | 4·43 | Oat straw | 1·29 |
| Wheat bran | 4·15 | Beet pulp | 0·23 |

From Table II which gives the lysine nitrogen content of milk and of some common feeds it is clear that 1 lb. of blood meal or meat meal will supply enough lysine to make 1 lb. of milk, while an equivalent amount of the vegetable feeding stuffs will be insufficient for the purpose. 11-34% higher intakes are necessary with beans, soya-beans and peas and 100% higher with peanut cake, wheat bran and cotton seed; with still others an increase of three-fold or more is necessary. The maintenance requirement of lysine is considered to be 4 g. per day for a 1000 lb. animal. Wright's work also indicates the necessity of taking into account seasonal variations in the amnio-acid content of grass with a consequent alteration in its nutritive value.

As wool is nearly pure keratin containing a high proportion of the sulphur-containing amino-acid cystine the question of the cystine requirement of sheep has attracted attention for a long

time. It was suggested by Robertson that the capacity of any territory for carrying sheep is determined by the content of cystine in its pasture grasses. The requirement of sheep for preformed cystine has not been estimated but it appears probable that this amino-acid can be synthesised by the sheep, for according to Rimington wool contains more cystine than can be accounted for by the amount in the feed. In contrast to this are the experimental result of Martin in Australia who found that blood meal fed to sheep on the range led to a 35% increase in wool production. It was also found that with 1 g. of cystine in the daily ration there was a 14% improvement in wool production and with 1 g. of cystine injected an increase of 34%. The disagreement with the results of Rimington and of Dutoit who found no such beneficial effect are probably to be explained on the basis of differences in the cystine content of the basal diets; the supplementary relation shown by Rose to exist between cystine and methionine may also play a part in this connection. As far as English pastures are concerned Pollard and Chibnall have shown that the pastures contain all the cystine required by sheep for wool production.

*Protein requirements as assessed by feed trials.* Experiments of this type mark the beginning of the scientific approach to the problem of protein in the nutrition of livestock. But as our knowledge is still very inadequate in husbandry today protein standards have to be based mainly on long-term feeding trials, such as have been largely carried out in America. In these feeding trials intakes of protein necessary for (a) maintenance of weight and general health and (b) for maximum milk production are determined by adding varying quantities of protein to an otherwise adequate diet. From such studies continued over long periods, sometimes for a whole generation, have been derived the standards usually adopted in America. The most widely accepted ones provide for a 1000 lb. dairy animal a daily intake of about 0·7 lb. of digestible crude protein plus a production ration containing from 1½ to 2 times as much protein as is produced in the milk i.e., from ·05 to ·09 lb. per lb. of milk. The corresponding energy requirements are 7·925 lb. of digestible materials for maintenance plus ·26 to ·5 lb. for each lb. of milk produced. These figures are nearly twice as high as those arrived at from nitrogen balance studies. Experiments have therefore been carried out to ascertain the effect of lower protein intakes. In a well-known investigation Cavy compared the effect of a diet with a protein intake as given above with another one in which ·5 lb. protein for maintenance plus 1·25 times the milk protein was fed. Two

good dairy cows were used in the experiment and the milk yield determined over successive lactation periods, the experimental diets being fed from the beginning of the previous dry period. When transferred from the high to the low protein diet one cow responded with a fall of 50% in milk production in the immediately succeeding lactation period. The other animal maintained its output for sometime, but at the expense of a 100 lb. loss in body weight; at the end of eight months both milk yield and fat content fell abruptly. In the second lactation period the milk production of this animal was reduced to 22% of that on the high protein diet. When restored to the high protein diet both animals resumed their normal production of milk and fat showing that no permanent injury had resulted from the low protein regimen. Experiments of this type make it clear that for maximum milk production the level of protein in the diet has to be much higher than is indicated by nitrogen balance studies. Several other lines of investigation also indicate the necessity for a high proportion of protein in the feed. Thus it was shown by Mitchell and Hamilton that in pigs not only nitrogen retention and rate of growth but also the utilization of energy improved with increase in protein intake. Similarly it was found by Morrison that in fattening pigs for the market on grain alone a 100 lb. increase in live weight required 700 lb. of grain, while with an adequate protein supplement half the quantity of grain was sufficient. Further, experiments in which animals were fed quantities of protein 30% higher than the savage standard (which is in itself a high protein ration) have dispelled the grounds for the belief in the harmful effects of high protein intake, although on economic grounds unnecessarily high levels of protein are to be avoided in practice.

*The Need for Increased Cultivation of Fodder Crops.* Undue weight cannot be attached to the absolute quantities of protein found desirable in these well-known American feed trials. The biological value of the proteins of feeds and therefore the quantities required will vary according to the amino-acid make up of the proteins present. It has also been suggested that different breeds of animals may differ in their ability to utilize the constituents of a ration so that feeding standards may be affected by geographical considerations. What is certain however is that a lactating animal cannot reach full efficiency in milk production on a ration in which the proportion of the energy derived from protein is less than about 9% of the total calories. This is usually expressed by saying that the nutritive ratio i.e., the ratio, Digestible Crude Protein : Total Digestible Carbohydrate + 2·5

x Digestible Fat should be less than 1 : 10. On feeds in which this ratio is a wider one a dairy animal cannot consume enough food to supply the quantity of protein required for efficient milk production. The higher the milk yield the narrower this ratio should be. The coarse dry fodders such as rice straw largely used in India in feeding milch cattle have a nutritive ratio of about 1 : 40 and require to be supplemented by protein rich concentrates in the form of oil-cakes to render them suitable for the purpose. According to an estimate made by Wright the total quantity of such concentrates available in India is barely sufficient to meet the needs of existing milk production. The increased cultivation of suitable fodder crops with a narrow nutritive ratio is therefore an essential preliminary condition for increasing the country's milk supply. Suitable fodder crops for this purpose are various grasses with a nutritive ratio just approaching 1 : 10 and more especially leguminous crops such as the clovers, lucerne and pulses which have a high protein content with nutritive ratios ranging from 1 : 4 to 1 : 10. As the growing of grasses depends upon the proper supply of nitrogenous nutrients in the soil and of the leguminous crops upon suitable conditions for the fixation of atmospheric nitrogen we are led to a consideration of nitrogen nutrition and metabolism in plants and the mechanism of nitrogen fixation by symbiotic bacteria.

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